Appendix

EFFICACY OF THE ANTI-CD22 RECOMBINANT IMMUNOTOXIN BL22 IN CHEMOTHERAPY-RESISTANT HAIRY-CELL LEUKEMIA

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ABSTRACT

Background Hairy-cell leukemia that is resistant to treatment with purine analogues, including cladribine, has a poor prognosis. We tested the safety and efficacy of an immunotoxin directed against a surface antigen that is strongly expressed by leukemic hairy cells.

Methods RFB4(dsFv)-PE38 (BL22), a recombinant immunotoxin containing an anti-CD22 variable domain (Fv) fused to truncated pseudomonas exotoxin, was administered in a dose-escalation trial by intravenous infusion every other day for a total of three doses.

Results Of 16 patients who were resistant to cladribine, 11 had a complete remission and 2 had a partial remission with BL22. The three patients who did not have a response received low doses of BL22 or had preexisting toxin-neutralizing antibodies. Of the 11 patients in complete remission, 2 had minimal residual disease in the bone marrow or blood. During a median follow-up of 16 months (range, 10 to 23), 3 of the 11 patients who had a complete response relapsed and were retreated; all of these patients had a second complete remission. In 2 of the 16 patients, a serious but completely reversible hemolytic–uremic syndrome developed during the second cycle of treatment with BL22. Common toxic effects included transient hypoalbuminemia and elevated aminotransferase levels.

Conclusions BL22 can induce complete remissions in patients with hairy-cell leukemia that is resistant to treatment with purine analogues. (N Engl J Med 2001; 345:241-7.)

ABOUT 2 percent of cases of leukemia are of the hairy-cell type.[1,2] Prominent features of the disease are splenomegaly, pancytopenia, and the presence of cells in the peripheral blood, spleen, and bone marrow with hair-like cytoplasmic projections.[3,4] Splenectomy is generally only palliative,[5] but interferon alfa produces a partial remission in 30 to 70 percent of patients and complete remission, often of short duration, in 5 to 10 percent.[6-10] The purine analogues pentostatin (2′-deoxycoformycin) and cladribine (2-chlorodeoxyadenosine) induce complete remissions in up to 85 percent of patients and partial responses in 5 to 25 percent. With such treatment, the rate of disease-free survival at eight years is 60 to 75 percent.[10-13] However, patients with cladribine-resistant disease have poor responses to other therapy. In the 20 percent of patients with a variant form of hairy-cell leukemia, there are high numbers of circulating hairy cells, and the response to pentostatin or cladribine is poor.[12,14]

Classic or variant hairy cells are virtually always strongly positive for CD22, an adhesion molecule expressed exclusively on B cells.[15-19] To target CD22-expressing cells, we designed a recombinant immunotoxin, RFB4(dsFv)-PE38 (BL22), that contains the variable domain (Fv) of the anti-CD22 monoclonal antibody RFB4.[20,21] The Fv is fused to a fragment of pseudomonas exotoxin called PE38, which contains domains responsible for cell death but lacks the domain necessary for cell binding (Fig. 1).[22] BL22 induced complete remissions in mice with a B-cell lymphoma and killed fresh human malignant B cells in vitro.[23,24] To assess the clinical activity of BL22, we studied patients with hairy-cell leukemia that was resistant to cladribine in a dose-escalation trial.

METHODS

Of the 31 patients with B-cell cancers who were enrolled in a dose-escalation trial of BL22, 16 had hairy-cell leukemia. All patients had circulating malignant cells that expressed CD22, adequate organ function, and an absence of high levels of neutralizing antibodies against BL22; all were resistant to standard chemotherapy; and all provided written, informed consent.

Between 0.2 and 4.0 mg of BL22, which was produced by the Developmental Therapeutics Program of the National Cancer Institute, was diluted in 50 ml of 0.2 percent albumin in 0.9 percent sodium chloride and administered as a 30-minute intravenous infusion every other day for a total of three doses. To diminish inflammatory adverse effects, all patients who received at least 40 μg of BL22 per kilogram of body weight also received 5 mg of infliximab, the monoclonal antibody to tumor necrosis factor α (TNF-α), per kilogram before and one week after the beginning of each cycle and 12.5 to 25.0 mg of rofecoxib, the nonsteroidal selective inhibitor of cyclooxygenase-2, per day. Patients without neutralizing antibodies who did not have progressive disease[24] could be treated again after restaging at intervals of three weeks or more. After a partial response, patients could receive a total of 16 cycles of BL22, and patients who had a complete response could receive 2 additional cycles. Patients could receive higher doses of BL22 during retreatment if these doses were found to be safe in patients who received them during the initial treatment.

The disease was assessed by computed tomography, flow cytometry to detect hairy-cell-leukemia antigens, and polymerase-chain-reaction (PCR) assay of nucleated blood cells to detect immunoglobulin heavy-chain monoclonality and by histologic examination of bone marrow. The criteria for complete remission were an absence of evidence of disease in radiographic studies and an absence of tumor cells in the bone marrow and peripheral blood according to morphologic criteria, as ascertained at least four weeks after the last dose of BL22.[25] The presence or absence of minimal residual disease in the biopsy specimen of bone marrow was determined

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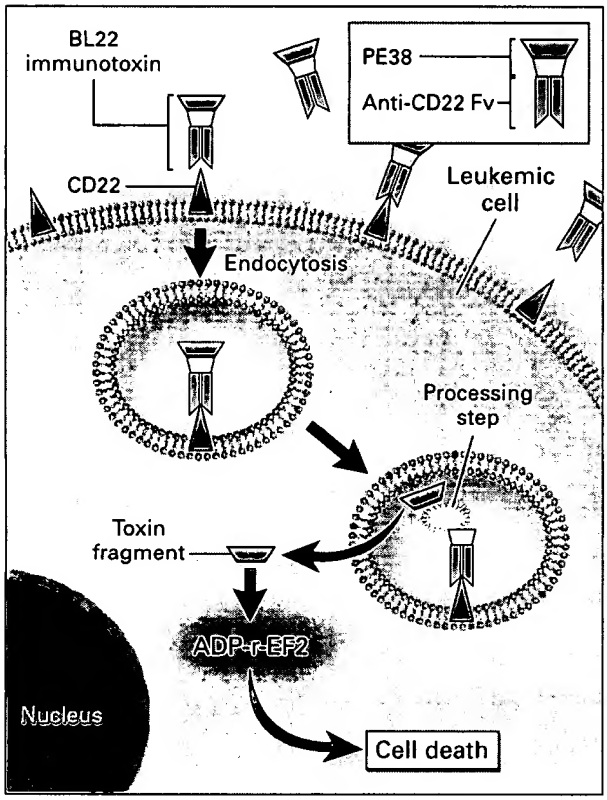

Figure 1. Action of BL22 on Hairy Cells.

BL22 binds to CD22, enters the cell by endocytosis, and is processed and translocated to the cytosol, where it catalyzes ADP ribosylation of elongation factor 2 (ADP-r-EF2) and causes the death of the leukemic cell.[22]

microscopically by immunohistochemical analysis[26] four weeks or more after the last dose of BL22 as well. Radiologic and biopsy evidence of complete remissions and determinations of whether minimal residual disease was present were independently reviewed by personnel who were unaware of the treatment status of the patients.

The maximal tolerated dose was the highest level at which dose-limiting toxic effects (at least grade 3 according to the Common Toxicity Criteria, version 2.0, with exceptions that are not considered to be dose-limiting[27]) occurred in none or one of six patients during the first cycle. Six patients received the maximal tolerated dose (three infusions of 40 μg per kilogram) in the first cycle. A cytotoxicity assay on Raji cells[27] was used to determine plasma levels of BL22 and neutralizing antibodies. The PCR assay used to detect monoclonal B cells (hairy cells) was not sequence-specific and was performed on DNA extracted from mononuclear cells with the use of primers for framework region 3 and the junctional region of the immunoglobulin heavy-chain gene.

RESULTS

Patients

The median age of the patients was 54 years, and the median time from diagnosis was 8 years (Table 1). Hairy cells from all patients were strongly positive for CD22, as measured by fluorescence-activated cell-sorter (FACS) analysis. Of the 16 patients, 13 had classic hairy-cell leukemia in which the cells expressed CD25, CD11c, and CD103 and had typical morphologic features. Three patients had variant disease[14] in which the leukemic cells lacked CD25 or CD103 (Patients 4, 7, and 11). Before they were enrolled in the study, the patients had received a median of three courses of treatment with a purine analogue (range, one to seven); all had received and were resistant to cladribine, as defined by an inadequate response (Table 1).

Dose Levels and Immunogenicity of BL22

BL22 contains a bacterial toxin that is expected to be immunogenic in humans. However, neutralizing antibodies against the toxin were generated in only 4 of the 16 patients (Patients 2, 5, 9, and 12); these antibodies were detected after cycles 4, 1, 2, and 4, respectively (Table 2). Patient 5 had a low level of neutralizing antibodies before receiving BL22 and had an anamnestic response during the first cycle. The limited immunogenicity of BL22 made possible the administration of repeated cycles.

Response to BL22

Of the 16 patients, 11 had a complete remission and 2 had a partial remission. These two patients are still receiving treatment. The remaining three patients received 2 or 6 μg of BL22 per kilogram or had neutralizing antibodies before therapy was started. Residual hairy cells were always strongly positive for CD22. Patients 2 and 5 had 98.0 and 99.5 percent reductions in circulating hairy cells, respectively, but had less than 50 percent decreases in the size of abdominal masses and were not retreated because of the presence of neutralizing antibodies (Table 2). In the 13 patients with a complete or partial response, the abnormal findings on radiography disappeared. Splenomegaly resolved in all eight patients whose spleens had not been surgically removed (Table 1). As Figure 2 shows, the absolute neutrophil count, platelet count, and hemoglobin level improved after treatment. Three patients (Patients 7, 9, and 15) had iron deficiency after treatment with BL22, which may have limited the improvement in hemoglobin levels.

As Table 3 shows, six patients had a complete remission after receiving only one cycle of BL22, whereas in five patients the remission occurred after two to nine cycles. In patients who had a response, the rapid reduction in circulating hairy cells was consistent with a direct cytotoxic effect of BL22. Levels of circulating malignant cells were measured by flow cytometry, which can detect levels of monoclonal B cells of 0.01 to 0.05 percent. In most patients there was more than a 90 percent reduction in such cells by day 3 of cycle 1 (the effect of a single dose) and more than a 99 percent reduction by day 8. Patients 9, 13, and 16 had no detectable monoclonal B cells by day 8. In Patients 4, 7, and 11, all of whom had the variant form of hairy-

TABLE 1. CLINICAL CHARACTERISTICS OF PATIENTS WITH HAIRY-CELL LEUKEMIA AT THE INITIATION OF TREATMENT WITH BL22.

PATIENT NO.	AGE (YR)/ SEX	YEARS AFTER DIAGNOSIS	PRIOR TREATMENT	CIRCULATING HAIRY-CELL COUNT	SITES OTHER THAN BLOOD OR MARROW WITH HAIRY CELLS
				cells/mm³	
1*	71/F	37	Splenectomy, interferon alfa, four courses of cladribine, two courses of pentostatin, one course of cladribine, rituximab	88,000	None
2*	54/M	18	Splenectomy, interferon alfa, one course of pentostatin, two courses of cladribine, anti-B4–blocked ricin, four courses of cladribine	690	Abdominal mass
3†	42/F	3	Three courses of cladribine	11	Spleen
4‡§	46/M	9	Interferon alfa, two courses of cladribine, two courses of pentostatin, fludarabine, fludarabine and chlorambucil, chlorambucil, rituximab, one course of pentostatin, interferon alfa	132,000	Spleen
5¶	54/M	25	Splenectomy, one course of cladribine, interferon alfa	180	None
6†	61/M	9	Two courses of cladribine, interferon alfa	1,330	Spleen
7‡	70/M	2	Two courses of cladribine, interferon alfa, one course of cladribine	32,000	Spleen
8‡	50/M	2	One course of cladribine	46	Spleen
9‡	45/F	11	Two courses of cladribine, interferon alfa, one course of pentostatin	22	Spleen
10*	59/M	25	Chlorambucil and prednisone, splenectomy, chlorambucil, interferon alfa, one course of cladribine, one course of pentostatin, one course of cladribine	4,700	None
11‡	56/M	2	One course of cladribine, splenectomy	44,000	Abdominal mass
12‡	43/M	5	Two courses of cladribine, prednisone, interferon alfa	9	Spleen
13‡	57/F	5	One course of cladribine, interferon alfa, splenectomy	38	None
14‡	37/M	0.5	One course of cladribine, rituximab	26	Spleen
15*	54/M	6	Two courses of cladribine	65	Spleen
16*	55/M	21	Splenectomy, three courses of cladribine	6	None

*The patient had no response to the last course of cladribine.

†The patient had a complete response to the last course of cladribine that lasted less than six months and then relapsed.

‡The patient never had a complete remission.

§The spleen was removed after the third course of BL22 to resolve coagulopathy related to hairy-cell leukemia.

¶Complete remission was never documented.

cell leukemia, the number of circulating hairy cells began to decrease within 24 hours after the first dose. All three patients with the variant form had never had a complete remission with chemotherapy but had a complete remission with BL22.

Minimal Residual Disease

The presence of minimal residual disease in the bone marrow, as detected by immunohistochemical analysis, is associated with relatively short durations of complete remission in patients with hairy-cell leukemia.[26] We assessed marrow-biopsy specimens using immunohistochemical analysis with CD20 (L26) and CD3 (Leu4) antibodies; only 1 of 11 patients in complete remission had evidence of minimal residual disease in the bone marrow (Table 3). Tests for the presence of monoclonal B cells by flow cytometry of blood were negative in 10 of the 11 patients who had complete remissions. A population of hairy cells too low to quantitate (less than 0.05 percent) was detected in one patient. PCR studies, which can detect levels of hairy cells of 0.001 to 0.1 percent, failed to detect monoclonal B cells in the peripheral blood in any of the 11 patients in complete remission.

Relapse

Of the 11 patients in complete remission, 3 (Patients 4 and 7, who had variant disease, and Patient 12, who had classic disease) relapsed 8, 12, and 7 months, respectively, after complete remission was achieved. Pa-

TABLE 2. DOSE LEVELS, IMMUNOGENICITY, AND DOSE-LIMITING TOXIC EFFECTS OF BL22 IN PATIENTS WITH HAIRY-CELL LEUKEMIA.

PATIENT NO.	DOSE*	ANTIBODY RESPONSE†	DOSE-LIMITING TOXIC EFFECTS
	μg/kg		
1	3		
2	6 (4 cycles), 10	Low level of activity after cycle 4	
3	10 (2 cycles), 20 (2 cycles)		
4	30 (9 cycles), 40, 50, 40 (3 cycles)		
5	30 (2 cycles)	High level of activity after cycle 1	Grade 3 hypotension, bone pain, vascular-leak syndrome with grade 1 weight gain
6	30 (3 cycles)		
7	30 (6 cycles), 40 (3 cycles)		
8	30 (2 cycles)		Hemolytic–uremic syndrome‡
9	40 (2 cycles)	High level of activity after cycle 2	
10	40 (2 cycles), 50, 40 (2 cycles)		
11	50 (2 cycles), 40		
12	50 (2 cycles), 40 (3 cycles)	Low and high levels of activity after cycles 4 and 5, respectively	
13	50 (2 cycles)		Hemolytic–uremic syndrome§
14	40 (15 cycles)		
15	40 (13 cycles)		
16	40 (2 cycles)		

*The amount of BL22 administered intravenously three times per cycle is indicated. Cycle 2 for Patients 5 and 7 was limited to 1 and 2 doses, respectively. Patients 4, 7, and 12 received 3 cycles, 4 cycles, and 3 cycles of BL22, respectively, after relapsing.

†High and low levels of neutralizing-antibody activity indicate over 75 percent and 25 to 75 percent neutralization of 1 μg of BL22 per milliliter of serum, respectively.

‡Toxic effects were characterized by an acute exacerbation of chronic pancreatitis, grade 3 thrombocytopenia, proteinuria, hemolysis, grade 2 hyperbilirubinemia, and grade 1 creatinine elevation.

§Toxic effects were characterized by grade 3 thrombocytopenia, proteinuria, hemolysis, and creatinine elevation and by grade 2 hyperbilirubinemia.

tient 12 had received only one cycle after entering a complete remission and was the only patient with a complete response who was found to have minimal residual disease on bone marrow biopsy. When retreated, Patients 4, 7, and 12 had another complete remission after one to three cycles; Patient 12 had had a response to retreatment.

Toxicity of BL22

BL22 caused a dose-limiting cytokine-release syndrome, defined by fever, hypotension, and myalgia or arthralgia, in Patient 5. This complication was temporally related to a secondary immune response to BL22. The level of TNF-α in this patient (112 pg per milliliter) was elevated (normal level, less than 5 pg per milliliter). To prevent the cytokine-release syndrome, Patients 8 through 16 were pretreated with rofecoxib and infliximab. In Patients 8 and 13, a serious but completely reversible hemolytic–uremic syndrome, confirmed by renal biopsy, developed after the last dose during cycle 2 (Table 2). These two patients required 6 to 10 days of plasmapheresis but not dialysis. Both patients remain in complete remission more than 11 and 16 months after treatment. Hematologic toxic effects (neutropenia, anemia, or thrombocytopenia) and decreases in the T-cell count were not observed in the other 14 patients. Less serious toxic effects included transient hypoalbuminemia, elevations in aminotransferase levels, nausea, myalgia, edema, and slight elevations in creatinine levels; all were reversible.

DISCUSSION

We found that a recombinant immunotoxin, BL22, is active in patients with hairy-cell leukemia that is resistant to treatment with purine analogues. BL22 induced a complete remission in 11 of 16 such patients (69 percent) and a partial remission in 2. The three patients who did not have a response probably received inadequate treatment because preexisting toxin-neutralizing antibodies were present or low doses of the immunotoxin were administered. A potential confounding variable was the use of inhibitors of TNF-α in some patients to prevent the cytokine-release syndrome. Although TNF-α is considered an autocrine growth factor for hairy cells and moderate antitumor activity has been reported with inhibition of TNF-α,[28] we believe it is unlikely that the use of infliximab or rofecoxib can account for our results, because four patients had already had a response before infliximab was administered. Of the 11 patients who had a complete response to BL22, 7 had never previously had a complete remission, 2 had had no response to their last course of therapy with a purine analogue, and 2 had had complete remissions lasting less than six months (Table 1). We are unaware of any other treatment, including interferon alfa, fludarabine, chlorambucil, and multiagent chemotherapy, that can produce a high rate of complete remission in patients with hairy-cell leukemia that is resistant to purine analogues. Complete remissions also occurred in all three patients with variant hairy-cell leukemia, which responds poorly to pentostatin[12] and cladribine.[14,29,30]

Up to 50 percent of patients who have a complete response to cladribine or pentostatin still have minimal residual disease detectable by immunohistochemical analysis of bone marrow; this finding portends a decreased rate of disease-free survival.[26,31,32] By contrast, minimal residual disease was present in the bone marrow of only 1 of our 11 patients who had a com-

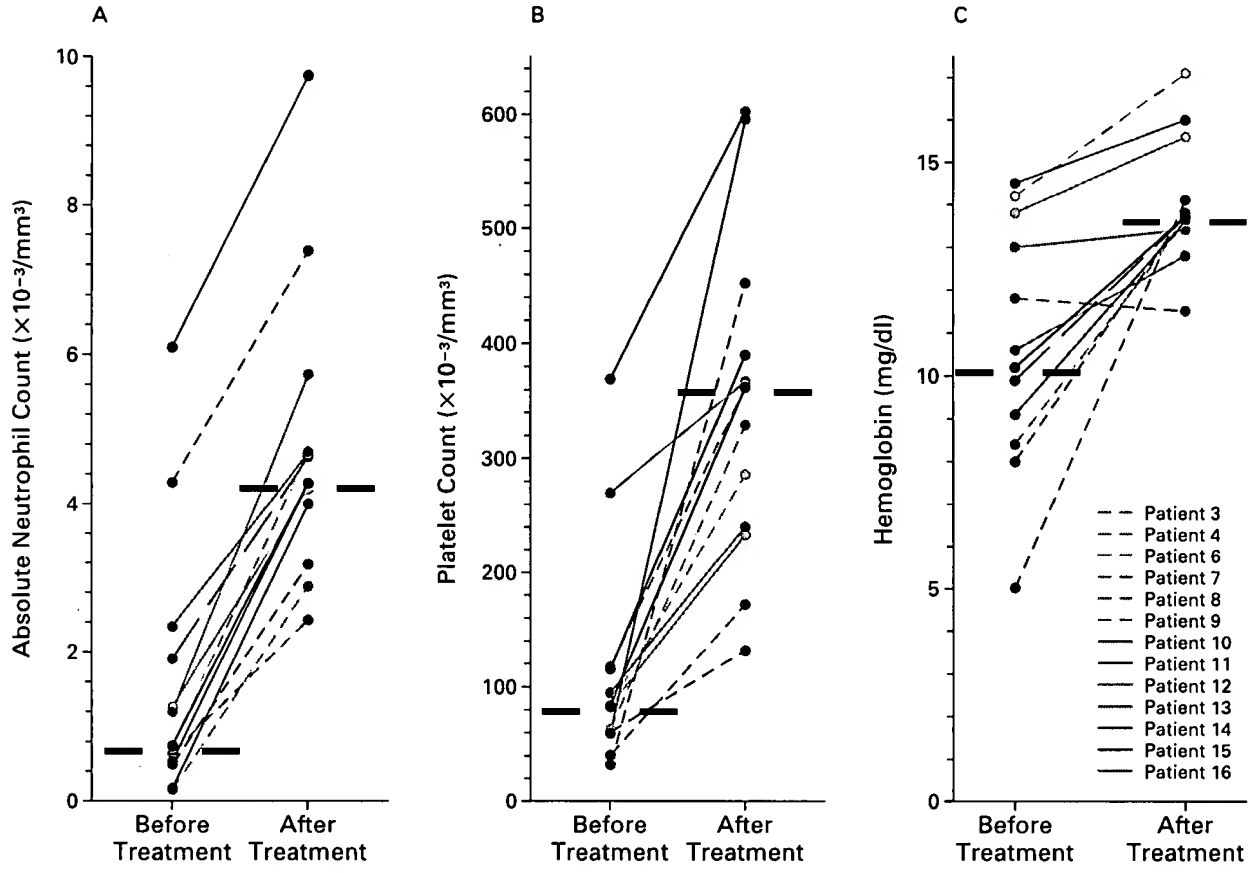

Figure 2. Response of Patients with Pancytopenia to BL22.

Improvement in the absolute neutrophil count (Panel A), the platelet count (Panel B), and the hemoglobin level (Panel C) is shown for Patients 3, 4, 6 through 13, and 16 after treatment with BL22 leading to complete remission and for Patients 14 and 15 after treatment leading to partial remission. Horizontal bars indicate median values. Differences between values measured before and after treatment with BL22 were significant ($P<0.01$ for Panels A and B and $P<0.004$ for Panel C, by the Mann–Whitney rank-order test).

plete response. Despite the elimination of minimal residual disease by purine analogues, hairy cells can be detected in the marrow by sensitive PCR techniques.[33,34]

The low level of toxicity of BL22 made possible the administration of multiple cycles to most patients. This is potentially a major advantage over cladribine and pentostatin, both of which cause considerable myelosuppression and long-term reductions in CD4+ T cells.[35,36] Although we were limited by our protocol to two cycles of consolidation therapy regardless of the presence or absence of minimal residual disease, additional cycles may be useful, particularly in patients with variant disease, if relapse is frequent after treatment with BL22. Additional follow-up of these patients and treatment of new patients will be necessary to determine whether there is a dose response.

We recently reported that the recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2), which contains an anti-CD25 Fv fused to a truncated pseudomonas exotoxin (PE38), induced one complete remission and three partial remissions in four patients with hairy-cell leukemia that was resistant to treatment with cladribine.[27,37] Like LMB-2,[19] BL22 is specifically cytotoxic to primary cultures of hairy cells (data not shown), suggesting that clinical responses are due to internalization of BL22 by malignant cells. The response of hairy-cell leukemia to these new agents suggests the potential for targeting other diseases with the truncated toxin PE38. Because CD22 is expressed in all patients with hairy-cell leukemia and at higher concentrations than CD25, BL22 is the preferable recombinant immunotoxin for the treatment of this disease.

The cause of hemolytic–uremic syndrome in two of our patients, both of whom recovered fully and had complete remissions, is unknown. Fatal hemolytic–uremic syndrome was reported with the combination of the immunotoxins RFB4–deglycosylated ricin A chain (directed against CD22) and HD37–deglycosylated ricin A chain (directed against CD19) known

TABLE 3. RESPONSE TO BL22 IN PATIENTS WITH HAIRY-CELL LEUKEMIA.*

PATIENT NO.	STANDARD CRITERIA			MINIMAL RESIDUAL DISEASE		
	RESPONSE	CYCLES BEFORE COMPLETE REMISSION	DURATION OF RESPONSE (MO)	BIOPSY OF BONE MARROW	FACS ANALYSIS OF BLOOD	PCR ANALYSIS OF BLOOD
1	Progressive disease	NA	NA	ND	+	ND
2	Marginal response	NA	4	ND	+	ND
3	Complete remission	2	>22	−	−	−
4	Complete remission	9	8, >3	−	−	−
5	Marginal response	NA	4	ND	+	ND
6	Complete remission	1	>20	−	−	−
7	Complete remission	4	12, >4	−	+	−
8	Complete remission	1	>6	−	−	−
9	Complete remission	1	>14	−	−	−
10	Complete remission	3	>12	−	−	−
11	Complete remission	1	>13	−	−	−
12	Complete remission	1	7, >2	+	−	−
13	Complete remission	2	>11	−	−	−
14	Partial remission	NA	>11	+	−	−
15	Partial remission	NA	>11	+	−	−
16	Complete remission	1	>11	−	−	−

*Marginal response indicates a 98.0 to 99.5 percent reduction in circulating hairy cells. Patients 4, 7, and 12 each had a second complete remission. Determinations of minimal residual disease were either positive (+), negative (−), or not determined (ND). FACS denotes fluorescence-activated cell-sorting, PCR polymerase chain reaction, and NA not applicable.

as Combotox,[38] suggesting a CD22-related mechanism.[39] Since we began taking precautions to prevent renal injury, including improving hydration and avoiding the use of intravenous contrast medium immediately before treatment with BL22, we have seen no other cases of hemolytic–uremic syndrome in 63 cycles of three doses of 40 to 50 μg per kilogram. Nevertheless, the hemolytic–uremic syndrome remains a serious potential complication; in our opinion, however, the responses to BL22 justify its testing in patients with a poor prognosis.

Unlike conjugates containing the deglycosylated ricin A chain,[20,21,40,41] the smaller BL22 molecule containing PE38 did not cause pulmonary edema, a serious complication of vascular-leak syndrome; this may have allowed patients with the hemolytic–uremic syndrome to recover. The absence of fatal vascular-leak syndrome with BL22 is consistent with the sensitivity of human umbilical-vein endothelial cells to the deglycosylated ricin A chain and their resistance to PE38.[42,43]

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Drs. FitzGerald and Pastan hold the partial patent rights to the invention of the truncated pseudomonas exotoxin.

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